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Clinicopathologic and Histopathologic Renal Abnormalities in Dogs with Coccidioidomycosis

L.R. Mehrkens, F.C. Mohr, and J.E. Sykes

Background: We observed evidence of protein-losing nephropathy in some dogs with coccidioidomycosis, suggestive of immune complex glomerulonephritis (ICGN). The goal of this study was to understand the prevalence of renal histopathologic lesions and proteinuria in dogs with coccidioidomycosis.

Hypothesis: Biochemical and histopathological evidence of glomerular lesions is present in dogs with coccidioidomycosis.

Animals: Hundred and fifty-six dogs with naturally occurring coccidioidomycosis.

Methods: Retrospective case series. Clinical information and results of clinicopathologic testing were retrieved from the University of California, Davis Veterinary Medical Teaching Hospital (VMTH). Microscopic sections of renal tissue procured from necropsy of dogs with coccidioidomycosis were examined to evaluate the nature and distribution of lesions.

Results: A total of 156 dogs with coccidioidomycosis were identified; 87 dogs had serum biochemistry and a urinalysis performed, 17 had urine protein:creatinine ratios (UPCs), and 24 had renal tissue available for histopathology. Eleven (13%) of the 87 dogs were azotemic, 55 (63%) were proteinuric (of which 14 [25%] had clinically relevant proteinuria defined as ≥3+ or ≥500 mg/dL), and 14 dogs had UPC ≥0.5 (range, 0.5–21.5, median 4.2). Thirteen (54%) of 24 dogs had renal histopathologic lesions suggestive of ICGN. Seven of these dogs had urinalyses performed; 5 (71%) had clinically relevant proteinuria as described above. Two dogs (33%) with normal glomeruli had granulomatous nephritis, 1 of which had intralesional *Coccidioides* spherules.

Conclusions and Clinical Importance: Coccidioidomycosis should be considered as a possible contributor to glomerular disease in dogs. Whether similar lesions occur in other mammalian hosts, including humans, warrants further investigation.

Key words: *Coccidioides immitis*; Immune complex glomerulonephritis; Proteinuria; Urine protein:creatinine ratio; Valley fever.

Coccidioidomycosis is the most common systemic mycosis in dogs the southwestern United States. Disease results from infection by the dimorphic, saprophytic fungal organism *Coccidioides immitis* or *Coccidioides posadasii*. Infection by *Coccidioides* spp. occurs in a wide range of host species, including humans, a myriad of domestic and exotic mammals, and, rarely, reptiles.[1] Also known as "valley fever", coccidioidomycosis is endemic in semiarid regions of California, Arizona, New Mexico, Texas, and Northern Mexico and semiarid regions in South America. Inhalation is the most common route of infection in both animals and humans and typically occurs after fungal hyphae have desiccated and matured into arthroconidia that are easily aerosolized. The arthrospores are inhaled and dispersed along the bronchiolar tree. Here, they undergo structural transformation into spherules, which enlarge and undergo endosporulation. Eventually, new endospores are released into the surrounding tissue and the cycle continues until the host is able to mount an appropriate immune response. Direct cutaneous inoculation of arthrospores has been reported, but these instances are rare and typically result in local granuloma formation.[2]

Infections caused by *Coccidioides* may be subclinical or result in severe illness and death. Dissemination to extrapulmonary organs is a potential sequela in some animals. One study indicated that 80% of dogs develop primary pulmonary infection whereas 20% develop disseminated disease.[3] Pulmonary coccidioidomycosis is characterized by clinical signs that include chronic cough, lethargy, and respiratory distress. Radiographic pulmonary lesions may be characterized by interstitial to nodular patterns, with hilar lymphadenomegaly. Dogs with disseminated disease have wide variation in clinical and clinicopathologic signs. Diagnosis by serology is straightforward, although seropositive, subclinically infected and seronegative, ill dogs have been documented.[4]

Because dogs and humans share a common environment, dogs may serve as sentinels of human infection.[5]

Abbreviations:

ICGN	immune complex glomerulonephritis
IF	immunofluorescence
LM	light microscopy
TEM	transmission electron microscopy
UPC	urine protein:creatinine ratio
USG	urine specific gravity

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Human infection has steadily increased over the last 10 years, and coccidioidomycosis is considered a re-emerging infectious disease.[6] Dogs come into close contact with soil, because of a closer proximity to the ground and digging behavior, and may provide a clearer delineation of broadly defined endemic regions. In this sense, rapid recognition of *Coccidioides* infection in dogs in a clinical setting may benefit human public health as well as minimize morbidity in other dogs.

We have observed that some dogs with disseminated coccidioidomycosis have concurrent biochemical evidence of protein-losing nephropathy, but to date this observation has not been well documented in the medical literature. Possible mechanisms include deposition of antigen-antibody complexes at the level of the glomerulus or renal damage caused by endospore formation and associated pyogranulomatous inflammation. Therefore, the goal of our current study was to characterize the renal lesions and associated biochemical abnormalities in dogs with disseminated *Coccidioides* infection.

Materials and Methods

Criteria for Selection of Cases

Electronic medical records of dogs examined at the University of California-Davis William R. Pritchard Veterinary Medical Teaching Hospital (VMTH) between 1990 and 2013 were searched for dogs with a diagnosis of coccidioidomycosis with the keyword ("coccid*"). Medical records were reviewed by 2 of the authors (LM and JS). Dogs were included in the study if they had a diagnosis of coccidioidomycosis either based on culture of *Coccidioides* spp. from tissues or body fluids; a serologic titer ≥1 : 2 by quantitative immunodiffusion, or through positive qualitative immunodiffusion with concurrent supportive clinical findings; or, identification of intralesional *Coccidioides* spherules by histopathology.

Procedures

Eighteen variables relating to signalment, history, and results of clinicopathologic testing and testing for other causes of proteinuria were extracted from the electronic medical record of each case. Not all variables could be obtained for every dog because diagnostic tests, including assays for comorbidities, were performed at the discretion of the primary clinician, based on the clinical abnormalities present at the time of evaluation. The age of each dog was recorded as the age at the time of diagnosis of coccidioidomycosis rounded to the nearest whole year. If dogs were <1 year old, age was recorded in months. Dogs were considered to have pulmonary coccidioidomycosis if they had clinical signs consistent with respiratory disease (eg, cough, dyspnea) and findings on thoracic radiography consistent with pulmonary coccidioidomycosis, in addition to a positive anti-*Coccidioides* antibody titer (defined as ≥1 : 2), identification of spherules in tissues obtained at biopsy or necropsy, or both. Dogs were considered to have disseminated coccidioidomycosis if they had radiographic or ultrasonographic findings consistent with disseminated coccidioidomycosis (ie, lytic or proliferative bone lesions, enlargement of abdominal lymph nodes, organomegaly) with a positive anti-*Coccidioides* titer, identification of spherules in non-respiratory tissues obtained at biopsy or necropsy, or both.

To identify dogs with renal disease, a subset of dogs was identified that either had (1) both a serum biochemistry panel and urinalysis performed, (2) kidney tissue available for histopathologic evaluation, either as a result of biopsy or necropsy, or both. Azotemia was defined as a serum creatinine concentration ≥1.4 mg/dL according to the International Renal Interest Society guidelines.[7] Isosthenuria was defined as a urine specific gravity (USG) of 1.008–1.012. Proteinuria was defined as the presence of any protein on urine dipstick or by photometry. For the purpose of our study, clinically relevant proteinuria was defined as 3+ or 4+ protein on urine dipstick or ≥500 mg/dL of protein as determined by photometry in the absence of bacteriuria, pyuria, and hematuria or a urine protein:creatinine ratios (UPC) >5. Dogs counted once for this purpose if they had both clinically relevant proteinuria on urinalysis and a UPC >5. Clinically relevant proteinuria was considered to be suspicious for the presence of underlying glomerular disease.

Renal Histopathology

Biopsy specimens were immersion fixed in 10% buffered formalin for histologic analysis. Paraffin-embedded tissues were sectioned at 5 μm thickness and stained with hematoxylin and eosin. Additional sections were prepared for staining with periodic acid Schiff, Masson's trichrome, or the Jones methenamine silver method. All microscopic sections were evaluated by a board-certified veterinary anatomic pathologist (FCM). Histologic features, in aggregate, that suggested a diagnosis of immune complex glomerulonephritis (ICGN) were hypercellularity in the peripheral aspects of glomerular lobules and the mesangium, capillary wall thickening, reduplication and remodeling, intracapillary neutrophils, nuclear pyknosis, and mesangial matrix expansion. Features for non-ICGN cases (excluding normal glomeruli) included mesangial hypercellularity, mesangial expansion, rare capillary wall thickening, and remodeling.

Results

Overall Dataset

A total of 156 dogs with coccidioidomycosis were identified. These ranged in age from 6 months to 13 years (median, 4 years). Fifty (32.1%) were castrated males, 41 (26.3%) were intact males, 54 (34.6%) were spayed females, and 11 (7.0%) were intact females. A total of 52 breeds were represented. The most commonly represented breeds were Labrador retrievers (n = 19), golden retrievers (n = 10), boxers (n = 8), Dalmatians and Dalmatian mixes (n = 8), border collies (n = 7), pitbull terriers (n = 6), weimaraners (n = 5), Hungarian vizslas (n = 5), and German shorthaired pointers (n = 5). Travel history was provided for 89 dogs. Thirty-three (37.1%) dogs had traveled to (or resided in) Arizona, 4 to Nevada (4.5%), and 35 (39.3%) to central California (San Joaquin Valley). Three dogs traveled to or resided in both Arizona and central California.

A specific diagnosis of coccidioidomycosis was made by serology with complement fixation (n = 105), gel immunodiffusion, or both and concurrent supportive clinical findings (n = 30), histopathology of biopsy specimens (n = 8), fungal culture (n = 12), cytologic examination of lavage fluids, aspirates, or impression smears (n = 2), and histopathology after necropsy (n = 12). In some cases, multiple methods were used to make a diagnosis. Antibody titers to *Coccidioides* ranged from 1 : 2

to 1 : 512 (median, 1 : 16). Other diagnostic tests performed included CBC (n = 136), serum biochemistry panel (n = 126), urinalysis (n = 88), aerobic bacterial urine culture (n = 32), thoracic radiographs (n = 137), abdominal ultrasound examination (n = 96), and necropsy (n = 24).

A total of 87 (55.8%) dogs had disseminated coccidioidomycosis and the remaining 69 (44.2%) had pulmonary coccidioidomycosis.

Biochemical Evidence of Renal Injury

A total of 87 dogs had both a serum biochemistry panel and urinalysis performed, 14 of which also had kidney tissue available for histopathologic examination. An additional 10 dogs had kidney tissue available for histopathologic evaluation but did not have both a serum biochemistry panel and urinalysis performed, and thus the total number of dogs for which histopathology was performed was 24. In these 24 dogs, kidney tissue for histopathology was obtained at necropsy.

Of all dogs included in study, 11 dogs (7.1%) had comorbidities identified at the time of diagnosis. These consisted of neoplasia (1 dog each with cutaneous mast cell tumor, histiocytic sarcoma, lymphoma, and myeloid leukemia), culture-negative infective endocarditis, medically controlled hypothyroidism, megaesophagus, canine monocytic ehrlichiosis, and Rocky Mountain spotted fever (1 dog each).

Of the 87 dogs that had both a serum biochemistry panel and urinalysis performed, 11 (12.6%) dogs were azotemic. In these 11 dogs, serum creatinine concentration ranged from 1.4 to 3.2 mg/dL (median, 1.6 mg/dL). The median USG for all azotemic dogs was 1.024. In the azotemic dogs, USG was <1.008 (1 dog), 1.008–1.012 (3 dogs), 1.013–1.020 (1 dog), 1.020–1.030 (3 dogs), and >1.030 (3 dogs). None of the azotemic dogs, and only 6 of the remaining 76 dogs that had both a serum biochemistry panel and urinalysis performed, were reported to have received fluid treatment before urine was collected.

A total of 55 (63.2%) of the 87 dogs had proteinuria on urinalysis, including all 11 azotemic dogs. Fourteen (25%) of these 55 dogs had clinically relevant proteinuria. Proteinuria was semiquantified by dipstick in 44 dogs, 15 (27.3%) of which had trace protein, 12 (21.8%) had 1+ protein, 5 (9.1%) had 2+ protein, 9 (16.4%) had 3+ protein, and 3 (5.4%) had 4+ protein. The remaining 11 dogs had urine protein determined in mg/dL with a photometer[a] and of these, 3 (5.4%) had 25 mg/dL, 3 (5.4%) had 75 mg/dL, 3 (5.4%) had 150 mg/dL, and 2 (3.6%) had 500 mg/dL of protein or greater in the urine. The UPC was determined for 17 dogs, 16 of which had proteinuria on urinalysis. Of those 17 dogs, 3 had a UPC <0.5, 3 had a UPC of 0.5–2.0, 5 had a UPC of 2.1–5.0 (range, 3.1–4.7), and 6 had a UPC >5.0 (range, 6.2–21.5). Two of the dogs with a UPC >5 were azotemic.

In the 21 dogs with azotemia or clinically relevant proteinuria, additional testing for comorbidities that might be associated with renal injury consisted of aerobic bacterial culture for urinary tract infections (12 dogs), immunofluorescent antibody (IFA) serology for *Ehrlichia canis* (2 dogs), in-clinic ELISA serology for vector-borne pathogens[b] (2 dogs), *Leptospira* serology by the microscopic agglutination test (1 dog), and ELISA for *Dirofilaria* antigen (1 dog). All of these diagnostic tests were negative.

One of the dogs in the study developed nephrotic syndrome and died after treatment for coccidioidomycosis. A necropsy was not performed. Although 8 other dogs had mild hypercholesterolemia, biochemical evidence of nephrotic syndrome (hypercholesterolemia and hypoalbuminemia in conjunction with edema or ascites) was not identified in any of the other dogs in the study.

Histologic Evaluation

Kidney sections from 7 of the 24 dogs that had renal histopathology performed were stained only with H&E. The remainder had additional stains applied. Thirteen (54.1%) of the 24 dogs had histopathologic findings suggestive of ICGN. Interstitial fibrosis was detected in 10 (41.7%) of the 24 dogs. Infarcts were noted in 2 dogs. Five dogs had glomerular mesangial hypercellularity (not consistent with ICGN), 1 of which was diagnosed with renal lymphoma by histopathology. The glomeruli of the remaining 6 dogs were normal by light microscopic criteria, but 2 had granulomatous nephritis (1 with intralesional *Coccidioides* spherules), 1 had multifocal lymphocytic and plasmacytic nephritis, and 1 had cortical interstitial and medullary fibrosis. Two of the 6 dogs with apparently normal glomeruli had histologically normal interstitial tissue.

Of dogs with glomerular lesions consistent with ICGN, 1 dog had pulmonary coccidioidomycosis and 12 had disseminated infections. Seven dogs with ICGN had urinalyses performed and 5 (71.4%) had clinically relevant proteinuria. The UPC had been performed antemortem in 3 dogs that had renal histopathology performed after necropsy and were increased in all 3 dogs (4.7, 6.2, and 21.5). All 3 of these dogs had lesion patterns consistent with ICGN. Eleven of the dogs with ICGN had serum biochemistry panels performed antemortem and of those, 2 dogs were azotemic. Two cases without glomerular lesions had proteinuria. One dog had interstitial nephritis with intralesional spherules (75 mg/dL proteinuria), and the other had systemic histiocytosis without renal involvement (3+ proteinuria, 3+ hemoprotein).

Discussion

To our knowledge, ours is the first study to describe biochemical and histopathological evidence of renal injury in dogs with coccidioidomycosis, including glomerular lesions suggestive of ICGN. Immune complex glomerulonephritis is a common form of glomerular disease in dogs, with 1 study finding that approximately half (48%) of all kidney tissue biopsied because of suspected glomerular disease had ICGN.[8] Underlying causes of ICGN in dogs include chronic infections with arthropod-borne pathogens (especially

Ehrlichia canis, *Babesia canis*, *Leishmania* spp., and *Dirofilaria immitis*), but chronic *Coccidioides* spp. infection has not yet been identified as a possible underlying cause.

In dogs that had a urinalysis and serum biochemistry panel performed, azotemia was identified in 13% of dogs and proteinuria in 63% of dogs with coccidioidomycosis. Azotemia and proteinuria are relatively insensitive and nonspecific measures of renal dysfunction, and there are limitations to the ability of the cutoffs used in our study to identify renal dysfunction, such as variation in reference ranges of serum creatinine concentration with body size and test methodologies.[7] Nevertheless, these findings together with the results of histopathology suggest that renal dysfunction is present in a proportion of dogs with coccidioidomycosis. Testing for other causes of protein-losing nephropathies was not consistently performed on dogs in this dataset and would be an appropriate goal for a prospective study. In reports of coccidioidomycosis in humans, laboratory evidence of impaired renal function is rarely described. In 1 study of 162 human patients with coccidioidomycosis, 27% of cases had an abnormal urinalysis, defined as the presence of proteinuria, pyuria, or hematuria.[9] The magnitude of proteinuria in these human patients was not reported. It is generally accepted that a UPC >2.0 suggests proteinuria resulting from renal (tubular or glomerular) pathology, and a UPC >5 is consistent with glomerular lesions. Although only 15 of 55 dogs with proteinuria in our study had UPCs performed, 5 had results consistent with glomerular proteinuria (UPC >5). Urine protein:creatinine ratios were not determined for 18 dogs that had proteinuria on urinalysis that was ≥2+ or ≥25 mg/dL, suggesting that moderate to severe proteinuria may be under-recognized.

In our study, glomerular lesions compatible with ICGN were identified in more than half (54%) of the dogs for which biopsy results were available. When a urinalysis was available, many of those dogs (71%) had proteinuria reported as 3+ or 4+ or at least 500 mg/dL. Biopsy samples from dogs should be evaluated with a combination of light microscopy (LM), transmission electron microscopy (TEM), and immunofluorescence (IF), as is the standard in human medicine.[8] Given the retrospective nature of our study, we were only able to evaluate biopsy samples by LM because tissue was not preserved for other diagnostic methods. Although not the gold standard, in 1 study, the sensitivity of LM alone to detect ICGN lesions was 94%, with a specificity of 77%.[10] This same study noted that in 25% of cases, TEM either reversed or helped clarify an initial LM diagnosis. Thus, although we could not always definitively characterize the lesions visualized as ICGN, the high sensitivity and relatively high specificity of LM suggest that our study results approximate the incidence of ICGN in our study population.

Pulmonary coccidioidomycosis was the only condition detected antemortem and at necropsy in 1 dog with ICGN. This finding suggests the possibility that persistent pulmonary coccidioidomycosis may stimulate an immune response that leads to glomerular damage and proteinuria. If ICGN can occur secondary to chronic pulmonary coccidioidomycosis, concurrent ICGN may be under-recognized if urinalysis is not performed as part of the diagnostic evaluation for coccidioidomycosis. More studies are needed to determine the prevalence and clinical relevance of proteinuria in dogs with both pulmonary and disseminated coccidioidomycosis.

In summary, we have documented evidence of glomerular lesions in a proportion of dogs with chronic coccidioidomycosis. Because ours was a retrospective, descriptive study, more research is required to determine whether there is a causative association between *Coccidioides* infection and glomerular lesions in dogs. Future directions for these results would include using IF to verify the presence of immune complexes in these samples with ICGN-like lesions, which would be ideal to confirm suspicions of ICGN. In the interim, dogs with coccidioidomycosis should be more thoroughly screened for proteinuria to initiate timely treatment. In addition, testing dogs with protein-losing nephropathies that have resided in *Coccidioides*-endemic areas for anti-*Coccidioides* antibodies has the potential to identify an underlying cause and therefore permit more effective treatment of proteinuric renal disease.

Footnotes

[a] Roche Urisys 1800 Reflectance Photometer, Roche Diagnostics, Indianapolis, IN

[b] 4Dx SNAP, IDEXX Laboratories, Portland, ME

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Conflict of Interest Declaration: Jane Sykes serves as Associate Editor for the *Journal of Veterinary Internal Medicine*. She was not involved in review of this manuscript.

Off-label Antimedication Declaration: Authors declare no off-label use of antimicrobials.

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